National Institute of Standards & Technology

Certificate

Standard Reference Material® 2246

Relative Intensity Correction Standard for Raman Spectroscopy: 830 nm Excitation

This Standard Reference Material (SRM) is a certified spectroscopic standard for the correction of the relative intensity of Raman spectra obtained with instruments employing 830 nm laser excitation. A unit of SRM 2246 consists of an optical glass that emits a broadband luminescence spectrum when excited at this laser wavelength. The relative spectral intensity of the glass luminescence has been determined through the use of a white-light, uniform-source, integrating sphere that has been calibrated for its irradiance at NIST. The shape of the mean luminescence spectrum of this glass is described by a mathematical expression that relates the relative spectral intensity to the wavenumber (cm^{-1}) expressed as the Raman shift from the excitation laser wavelength. This model, together with a measurement of the luminescence spectrum of the standard, can be used to determine the spectral intensity response correction that is unique to each Raman system. The resulting instrument intensity response correction may then be used to obtain Raman spectra that are largely free from instrument-induced spectral artifacts.

This SRM is the sixth in a series of SRMs (2241, 2242, 2243, 2244, and 2245) that provides relative intensity corrections for Raman spectrometers employing lasers commonly used for Raman spectroscopy.

Certification: The log-normal model parameters describing the mean relative luminescence spectrum of SRM 2246 and associated confidence and prediction curves are given in Table 1.

Expiration of Certification: The certification of **SRM 2246** is valid, within the measurement uncertainty specified, until **30 September 2022**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Handling, Storage, and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Production and certification of this SRM were performed by S.J. Choquette and A.A. Urbas of the NIST Biosystems and Biomaterials Division and J.R. Anderson of the NIST Fabrication Technology Division. The SRM units were cut and polished by A. Kirchhoff of the NIST Fabrication Technology Division.

Statistical consultation was provided by D.V. Samarov of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

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Table 1. Coefficients of the certified (CERT), linearly shifted log-normal model[(a,b)] defined in Equation 1 that describes the mean luminescence spectrum of SRM 2246 for 830 nm excitation, and coefficients of the analytical approximations (with the same functional form as the model itself) to the upper and lower envelopes of the 95 % confidence (L_{CONF}, U_{CONF}) and 95 % prediction (L_{PRED}, U_{PRED})[(c)] bands depicted in Figure 1 (includes the results of the Type A and Type B evaluations of uncertainty)

	L_{PRED}	L_{CONF}	CERT	U_{CONF}	U_{PRED}
H	9.9148 E –01	9.8141 E –01	**9.9218 E –01**	1.0037 E +00	9.9287 E –01
w	3.0873 E +03	3.0887 E +03	**3.0853 E +03**	3.0838 E +03	3.0832 E +03
ρ	9.6168 E –01	9.6230 E –01	**9.6188 E –01**	9.6078 E –01	9.6207 E –01
x_0	2.3235 E +03	2.3234 E +03	**2.3237 E +03**	2.3231 E +03	2.3238 E +03
m	1.2699 E –05	1.2545 E –05	**1.2630 E –05**	1.3205 E –05	1.2561 E –05
b	–6.7285 E –02	–2.2739 E –02	**–2.1142 E –02**	–2.1496 E –02	2.4999 E –02

(a) The consensus (CERT) curve coefficients are for an unweighted log-normal model fit to the response data from three spectrometers. The uncertainty curve coefficients (CONF, PRED) are for log-normal model fits to 95 % confidence and 95 % prediction band expanded uncertainties, combining uncertainty components evaluated by Type A and Type B methods, with a coverage factor of $k = 2$, following the ISO/JCGM Guide [2].

(b) Where $I_{SRM}(\Delta\upsilon) = H \cdot e^{\left[\frac{-\ln 2}{(\ln\rho)^2}\left(\ln\left[\frac{(\Delta\upsilon - x_0)(\rho^2-1)}{w\cdot\rho}+1\right]\right)^2\right]} + m\cdot\Delta\upsilon + b$;

for $\Delta\upsilon$ = **110 cm^{-1} to 3000 cm^{-1}** Raman Shift relative to 830 nm excitation.

$I_{SRM}(\Delta\upsilon)$ has units of photons per second per square centimeter per wavenumber.

(c) The prediction band is one way of expressing the measurement uncertainty associated with the certified value: a measurement of this SRM over the range of Raman shifts considered in this certificate, made by a competent laboratory using a method comparable to those used at NIST to produce the certified value, is very likely (probability approximately 95 %) to lie within the prediction band.

Certified Values: A NIST certified value [1] represents a value derived from data reported in an SRM certificate for which NIST has the highest confidence in its accuracy to the extent that all known or suspected sources of bias have been fully investigated or taken into account. The measurand is relative luminescence measured as a function of Raman shift (cm^{-1}) from the laser excitation wavelength of 830 nm. Metrological traceability is to the NIST spectral radiance scale.

The certified values of the coefficients of the model describing the mean shape of the luminescence spectrum of SRM 2246, excited at 830 nm, are listed in Table 1. The spectrum and its associated expanded uncertainty (confidence) and prediction bands (for 95 % coverage probability) are shown in Figure 1.

The dependent variable of this model is the relative spectral intensity of the luminescence. The independent variable of this model is the wavenumber expressed in units of Raman shift (cm^{-1}) from the laser excitation wavelength of 830 nm. This model is certified to describe the luminescent response of the SRM when it is measured in the temperature range of 20 °C to 25 °C. This model certifies the shape of the luminescence spectrum between 110 cm^{-1} and 3000 cm^{-1} Raman shift for excitation with an 830 nm laser.

Certification Uncertainty: The combined standard measurement uncertainty comprises one set of components evaluated by application of statistical methods (Type A evaluation) and one set of components evaluated by other methods (Type B evaluation), combined in root sum of squares.

The Type A uncertainties include contributions attributable to differences between spectrometers used in the certification process and contributions from uncontrolled experimental factors, all of which find expression in the dispersion of the values of luminescent intensity that were obtained experimentally.

The Type B uncertainties includes contributions from the uncertainty in the white-light, uniform-source, integrating sphere irradiance calibration, assessed at 1 % (expanded uncertainty for 95 % coverage probability) of the certified value. Careful measurements of the glass have shown it to be spatially homogeneous in spectral luminescence. No significant changes in the shape of the luminescence spectrum occur over the range of laser power densities commonly used in Raman instruments.

The certified model was obtained by unweighted nonlinear least squares fitting the log-normal model of Equation 1 to measured values of $I_{SRM}(\Delta\upsilon)$ made by three different spectrometers. The associated uncertainty curves combine components evaluated by Type A methods and Type B methods, combined according to the ISO/JCGM Guide [2]. The Type A evaluations were performed using a Monte Carlo method consistent with the Supplement 1 to that Guide [3], and by application of standard methods for uncertainty analysis for linear and nonlinear statistical models [4,5].

Information Values: A NIST information value [1] is considered to be a value that will be of interest and use to the SRM user, but insufficient information is available to assess the uncertainty associated with the value.

The model describing the mean shape of the luminescence spectrum of SRM 2246, evaluated over the additional range of 3000 cm^{-1} to 5000 cm^{-1} Raman shift for excitation with an 830 nm laser, is provided as an information value. The model coefficients over this range are identical to the certified values of the coefficients provided in Table 1. The certified model was fit to data between 110 cm^{-1} and 5000 cm^{-1} Raman shift; however, the model was not certified beyond 3000 cm^{-1} Raman shift due to data limitations. The information value model, evaluated over the full range to which data was fit, 110 cm^{-1} to 5000 cm^{-1} Raman shift, is shown in Figure 2.

Physical Description: SRM 2246 is a chromium-doped (0.30 % mole fraction) oxide in a borosilicate-matrix glass. Each unit of this SRM consists of a glass slide that is approximately 10 mm in width × 10 mm in length × 1.65 mm in thickness, with one surface optically polished and the opposite surface ground to a frosted finish using a 400 grit polish. Two mounts are furnished with the slide. One is a 12.5 mm square cuvette-style optical mount. This mount is designed for the typical 12.5 mm sampling accessories widely used in chemical spectroscopy, i.e., absorbance, fluorescence, etc. The glass slide is retained, frosted side out, in a slot on the front face of the holder. Two plastic springs, also retained in the slot, hold the glass slide in place while allowing for positioning within the slot to accommodate different beam heights. Grooves on the sides of the slot require that the glass slide and springs be loaded into the slot from the bottom of the holder. The other mount is a 2.5 cm × 7.6 cm × 0.3 cm microscope slide-style holder. On the top face is a rectangular slot to retain the glass slide over a circular aperture in the center of the holder. Two plastic springs serve to hold the glass slide in the slot centered over the circular aperture. Removal of the SRM glass for measurements that are physically hindered by the holders does not alter the certified properties of this SRM.

Measurement Conditions: The certification measurements of the luminescence spectrum of SRM 2246 were made using three spectrometer systems: one commercial Raman microscopy system operated in a 180° backscatter geometry, one Raman system based on a 460 mm focal-length spectrograph designed for array detectors operated in a 180° backscatter geometry, and one commercial near-infrared (IR) spectrofluorometer operated in a 90° geometry. Excitation laser sources included an argon-ion-laser-pumped, titanium:sapphire, solid-state, tunable laser (830.00 nm ± 0.01 nm) and a frequency-stabilized 830 nm diode laser source (830.0 nm). Longpass edge filters were utilized for Rayleigh rejection on all systems. The absolute wavenumber axis of each spectrometer was calibrated using emission lines from low-pressure pen lamps operated with DC power supplies. The y-axis (relative spectral intensity) of each system was calibrated with a white-light, uniform-source, integrating sphere that had been calibrated for irradiance at NIST or a high-emissivity black body radiator with furnace temperature calibrated by the manufacturer. All certification data were acquired at nominal room temperature (22 °C).

INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

Handling and Storage: When not in use, the SRM should be stored in the container provided or in one providing comparable mechanical protection. Although not recommended, the glass standard may be removed from its mount without altering the certified properties of the glass.

Use: SRM 2246 is used to provide Raman spectra corrected for relative intensity. This requires a measurement of its luminescence spectrum on the Raman instrument and then a mathematical treatment of both this observed luminescence spectrum and the observed Raman spectrum of the sample. This SRM is intended for use in measurements in the temperature range of 20 °C to 25 °C.

For proper use of this procedure, attention must be paid to the following experimental conditions. The spectrometer laser and x-axis should be calibrated using the manufacturer's recommended methods. Validation of the Raman shift axis may be accomplished by referring to ASTM E1840-96 [6]. It is important that the laser excitation and emission collection system be focused on the frosted surface of the glass. The shape of the spectral luminescence will have some sensitivity to the placement of the glass surface relative to the collection optics of the spectrometer, which is minimized by scattering from the frosted surface. Measurement conditions should be arranged to furnish a spectrum of optimum signal-to-noise ratio of the SRM. The luminescence spectrum must be acquired over the same Raman range as that of the sample.

The relative intensity of the measured Raman spectrum of the sample can be corrected for the instrument-specific response by a computational procedure that uses a correction curve. This curve is generated using the certified model and the measured luminescence spectrum of the SRM glass. For the spectral range of certification, $\Delta\upsilon$ = 110 cm^{-1} to 3000 cm^{-1}, compute the elements of the certified relative mean spectral intensity of SRM 2246, $I_{SRM}(\Delta\upsilon)$, according to

$$I_{SRM}(\Delta\upsilon) = H \cdot e^{\left[\frac{-\ln 2}{(\ln\rho)^2}\left(\ln\left[\frac{(\Delta\upsilon - x_0)(\rho^2-1)}{w\cdot\rho}+1\right]\right)^2\right]} + m\cdot\Delta\upsilon + b \tag{1}$$

where $(\Delta\upsilon)$ is the wavenumber in units of Raman shift (cm^{-1}), H is peak height, w is peak width, ρ is half width ratio, x_0 is a location parameter for the log-normal profile, while m and b are the slope and intercept terms, respectively, for the linear term, with values as listed in Table 1. The elements of $I_{SRM}(\Delta\upsilon)$ are obtained by evaluating Equation 1 at the same data point spacing used for the acquisition of the luminescence spectrum of the SRM and of the Raman spectrum of the sample. $I_{SRM}(\Delta\upsilon)$ has been normalized to unity and is a relative unit expressed in photons per second per square centimeter per wavenumber**.** The data sets that are the measured glass luminescence spectrum, $S_{SRM}(\Delta\upsilon)$, and the measured Raman spectrum of the sample, $S_{MEAS}(\Delta\upsilon)$, must have the units of Raman shift (cm^{-1}). The elements of the correction curve $I_{CORR}(\Delta\upsilon)$, defined by Equation 2, are obtained from $I_{SRM}(\Delta\upsilon)$ and the elements of the glass luminescence spectrum, $S_{SRM}(\Delta\upsilon)$, by

$$I_{CORR}(\Delta\upsilon) = I_{SRM}(\Delta\upsilon) / S_{SRM}(\Delta\upsilon). \tag{2}$$

The elements of the intensity-corrected Raman spectrum, $S_{CORR}(\Delta\upsilon)$, are derived by multiplication of the elements of the measured Raman spectrum of the sample, $S_{MEAS}(\Delta\upsilon)$, by the elements of the correction curve [7]

$$S_{CORR}(\Delta\upsilon) = S_{MEAS}(\Delta\upsilon) \bullet I_{CORR}(\Delta\upsilon). \tag{3}$$

The Table 1 coefficients are **certified for use between 110 cm^{-1} and 3000 cm^{-1}.** The certified model is intended as a simple numerical descriptor of the spectral response observed over the wavenumber range studied. It is not claimed to be physically meaningful. **Extrapolation of the model outside the certification limits of 110 cm^{-1} and 3000 cm^{-1} is not a supported use of this SRM**. However, the model coefficients listed in Table 1 were fit to data spanning the Raman shift range from 110 cm^{-1} to 5000 cm^{-1}. The curve from 3000 cm^{-1} to 5000 cm^{-1} is not certified but provided only as an information value. The model cannot be used to extrapolate outside the 110 cm^{-1} to 5000 cm^{-1} limits without incurring significant error.

Use of this SRM at temperatures other than the certification temperature is not currently supported.

This SRM is not intended for use as a standard for the determination of absolute spectral irradiance or radiance.

This SRM was shown to be photostable under extended exposure to approximately 50 mW of 830 nm laser light focused through a 50X magnification microscope objective. While it is expected that higher power densities could be used without incurring damage, this is not recommended.

Luminescence Spectrum on the Wavelength Scale: The equation describing the mean luminescence spectrum of the glass SRM is given in Equation 1, where $\Delta\upsilon$ is the Raman shift in units of wavenumbers (cm^{-1}). For correction of spectra where the x-axis is in wavelength with units of nanometers, the same model coefficients can be used to calculate $I_{SRM}(\lambda)$ through the following transformation:

$$I_{SRM}(\lambda) = \left[\frac{10^7}{\lambda^2}\right] \cdot \left(H \cdot e^{\left[\frac{-\ln 2}{(\ln\rho)^2}\left(\ln\left[\frac{(z - x_0)(\rho^2-1)}{w\cdot\rho}+1\right]\right)^2\right]} + m\cdot z + b \right) \tag{4}$$

where

$$z = 10^7 \cdot [(1.0/\lambda_L) - (1.0/\lambda)] \tag{5}$$

and λ_L is the wavelength of the laser in nanometers and λ is the wavelength in nanometers. The prefactor of 10^7 in the first term of Equation 4 is required only if it is desired to preserve the numerical value of spectral areas computed relative to the two x-axis coordinate systems.

For user convenience, a spreadsheet containing the coefficients of the certified model, confidence band and prediction interval as well as an example relative spectral intensity correction calculation can be obtained at https://www-s.nist.gov/srmors/view_datafiles.cfm?srm=2246.

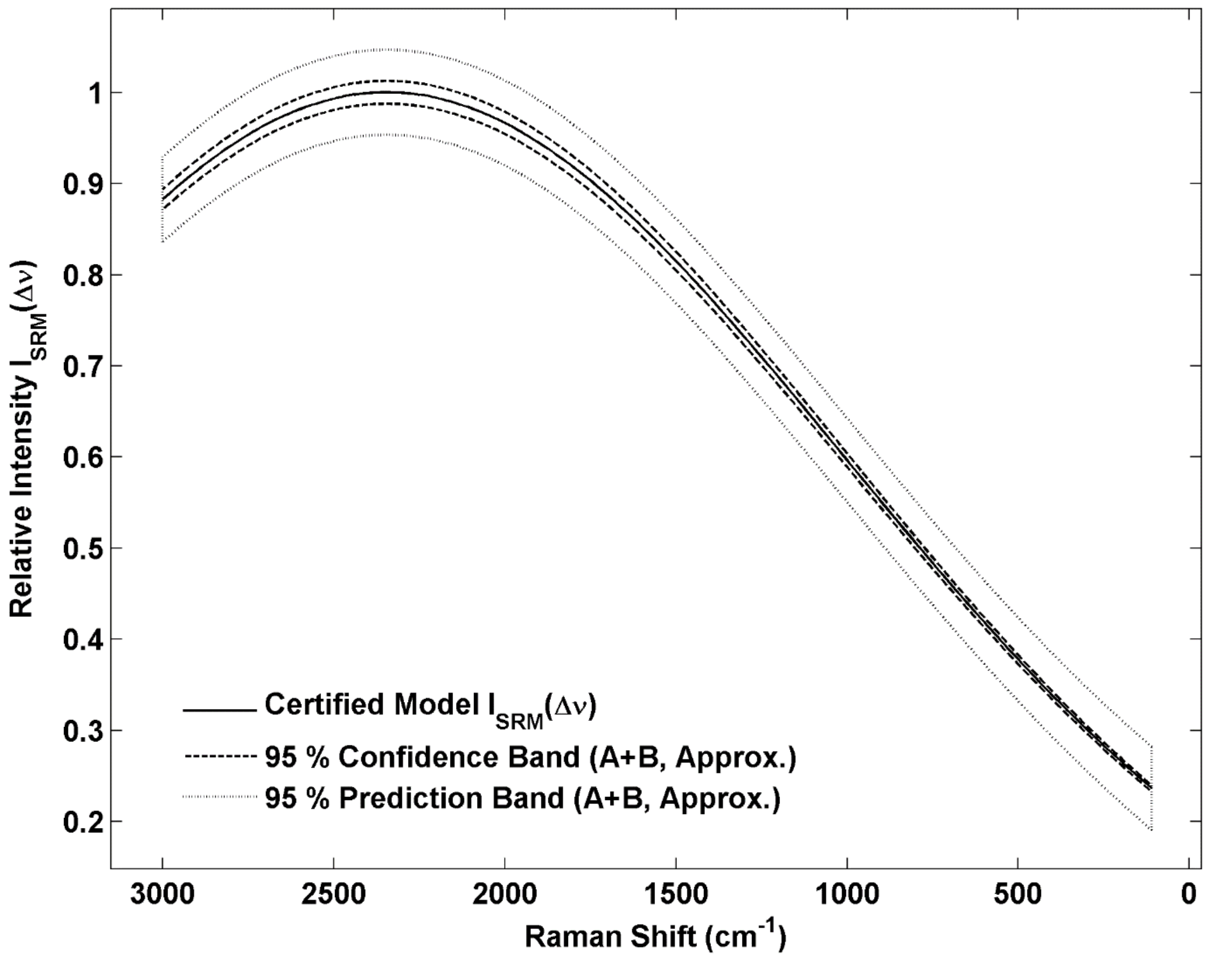

Figure 1. Certified, linearly shifted log-normal model describing the luminescence spectrum of SRM 2246 when excited at 830 nm. The horizontal axis has dimensions of Raman shift (cm^{-1}). The vertical axis is on a relative scale and normalized to unity with the dimensions of number of photons per second per square centimeter per wavenumber. The dashed lines (---) represent an approximate 95 % confidence band for the spectrum as a whole, reflecting components of uncertainty that have been evaluated by statistical methods (Type A) and by other methods (Type B). The wider, dotted lines (···), are the corresponding 95 % prediction band for individual spectra measured using methods and instruments comparable to those that were used in the certification.

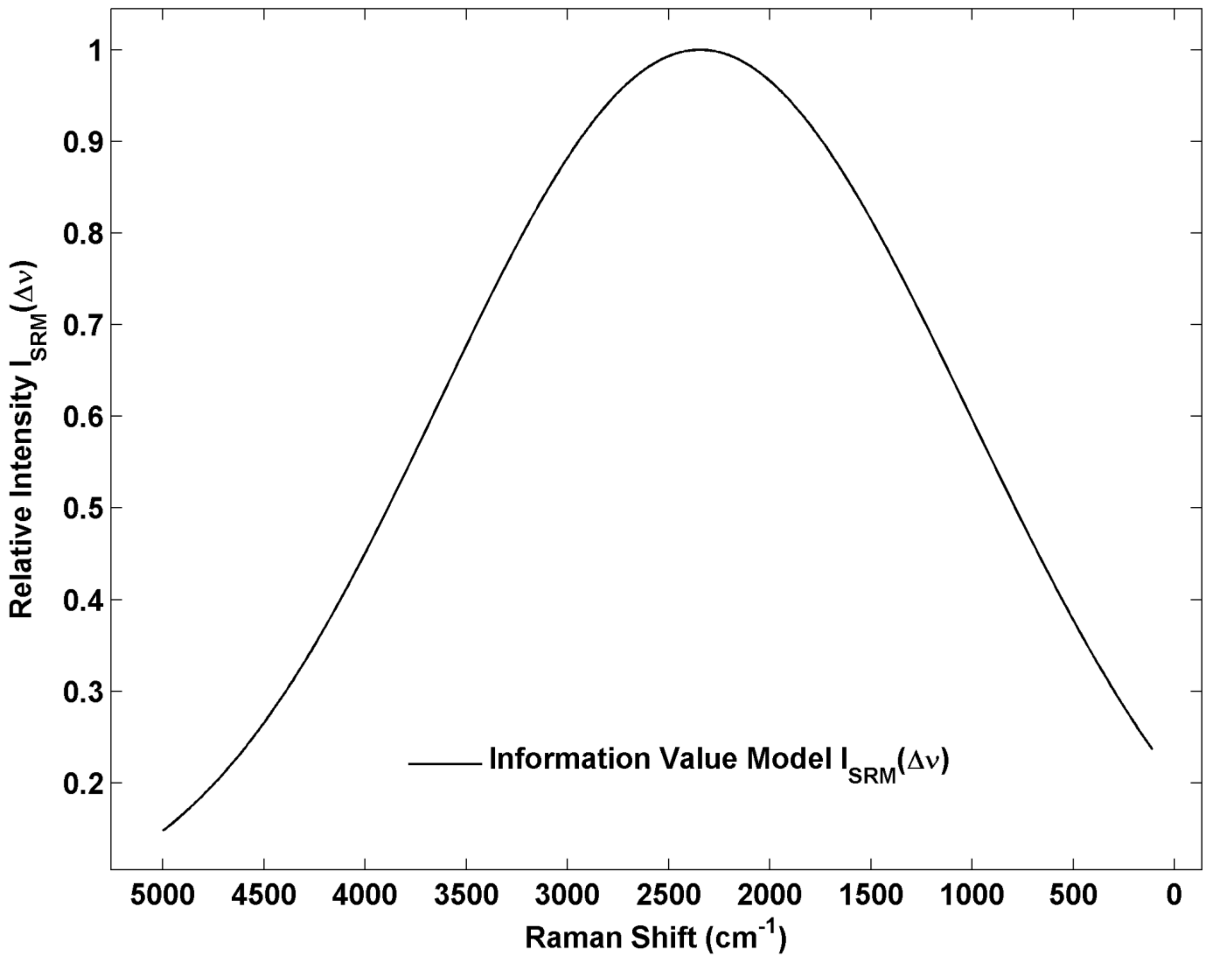

Figure 2. Linearly shifted log-normal model describing the luminescence spectrum of SRM 2246 when excited at 830 nm evaluated over the full range to which data was fit, 110 cm^{-1} to 5000 cm^{-1} Raman shift. The model fit from 3000 cm^{-1} to 5000 cm^{-1} is provided as an information value model. The horizontal axis has dimensions of Raman shift (cm^{-1}). The vertical axis is on a relative scale and normalized to unity with the dimensions of number of photons per second per square centimeter per wavenumber.

REFERENCES

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Certificate Revision History: 16 December 2015 (Addition of parenthesis to equation four, alternate wavelength models; editorial changes); 31 August 2012 (Original certificate date).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730, email srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.